LKB
BROMMA

Instruction Manual

LKB 2238
Uvicord® SII

90 01 3527

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Please read this manual carefully before attempting to use this instrument.

This manual describes the LKB 2238 Uvicord S II. The sections deal with the appearance and usage of this unit and contain information regarding installation and operation.

If you have any comments on this manual, we will be pleased to receive them at:

LKB-Produkter AB
Box 305
S-161 26 BROMMA
Sweden

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I Introduction

LKB 2238 Uvicord S II is a compact single beam UV-monitor with flow-through cell for monitoring the eluants from various column separations. Available wavelengths, selected by a combination of lamp and interference filter are 206, 226, 254, 280, 313, 365 and 405 nm.

LKB Uvicord S II is housed up in one combined optical electronic unit. This detector simultaneously produces two absorbance outputs – one fixed at 2AUFS and one selectable from 0.005 – 2AUFS in 9 steps. Thus, with the selection of the appropriate absorbance range, Uvicord S II enables monitoring of both high and low concentration substances simultaneously with a 2-channel recorder.

Operating at 206 nm it is possible to monitor many substances which are undetectable or poorly detectable by conventional UV-monitors. Substances such as non-aromatic peptides, amino acids, lipids nucleotides and certain carbohydrates have strong absorption at 206 nm whereas at higher wavelengths they show little or no absorption. Furthermore, most proteins also exhibit greater absorption at 206 nm than they do at 280 nm. By employing all fully integrated electronics and the very latest detection principles Uvicord S II performs to specification at ambient temperature and under cold conditions.

II Physical Description

Basic principle (see Block Diagram, Fig. 1)

Light from the high frequency excited gas discharge lamp is filtered by the interference filter before it is passing the measuring cell. The light transmitted through the measuring cell is the focused light on the measuring detector and converted to an electrical signal. This signal is directly proportional to the light intensity and is amplified by the following linear amplifier, the gain of which is controlled by the "Zero" adjust potentiometer.

Filtered light also falls on the Reference Detector. The reference signal is then amplified in the reference amplifier. This signal is directly proportional to the light intensity. It is used to control the lamp driver for obtaining constant and stable light from the lamp. The reference signal is also connected to the Logarithmic Converter. The Log-Converter receives both the reference signal and the measured signal. The ratio between these two signals is transformed by the converter and conveyed to the two output amplifiers. One of the Output Amplifiers has preset fixed gain which produces 1 volt and 100 mV outputs corresponding to 2 Absorbance Units. The other output amplifier has its gain controlled by the range switch. The full scale output signal from this amplifier is 100 mV which corresponds to a full scale absorbance of 0.005 to 2 Au, depending upon the range switch setting. This output signal is also fed through the output filter which time constant is selectable by a front panel switch.

A zero indicator connected to the output signal is used to adjust the baseline to a reading close to the zero volt. The final zero adjustment is then carried out on the recorder.

In order to ensure stable emission and long lamp life the lamp holder is thermostated. The thermostat is set to switch on heating when the lamp temperature goes below ambient temperature (>22°C).

Fig. 1

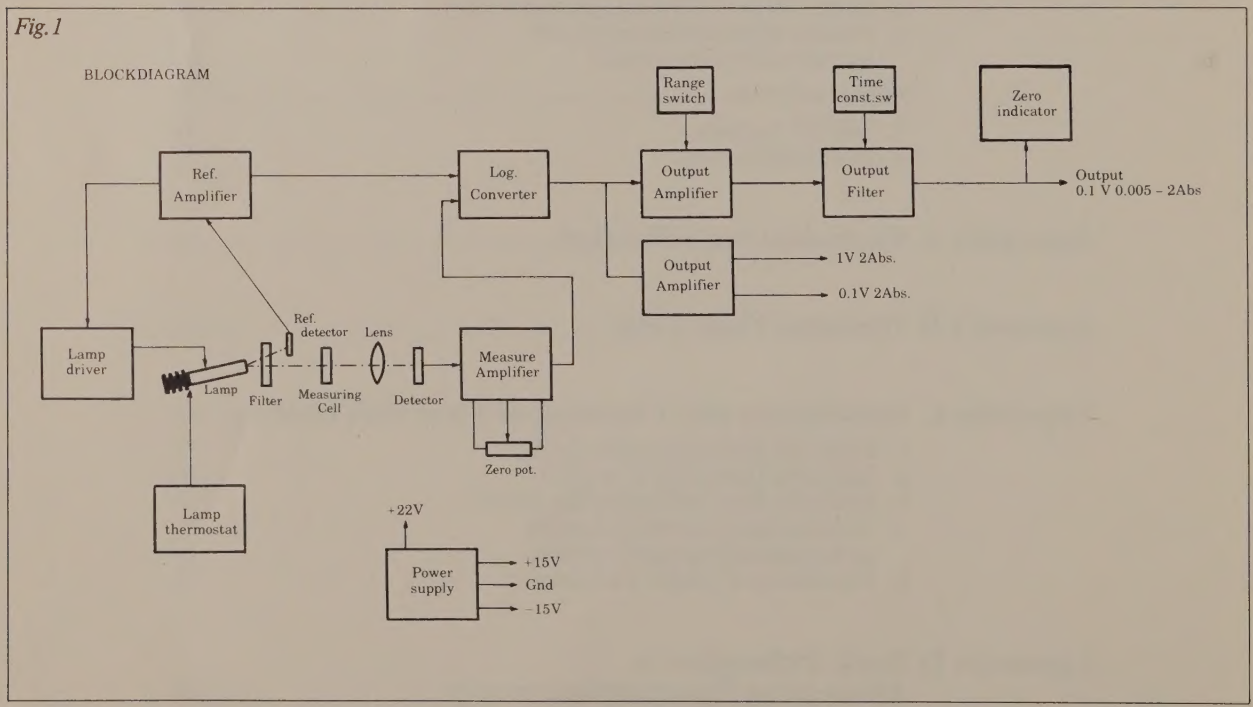
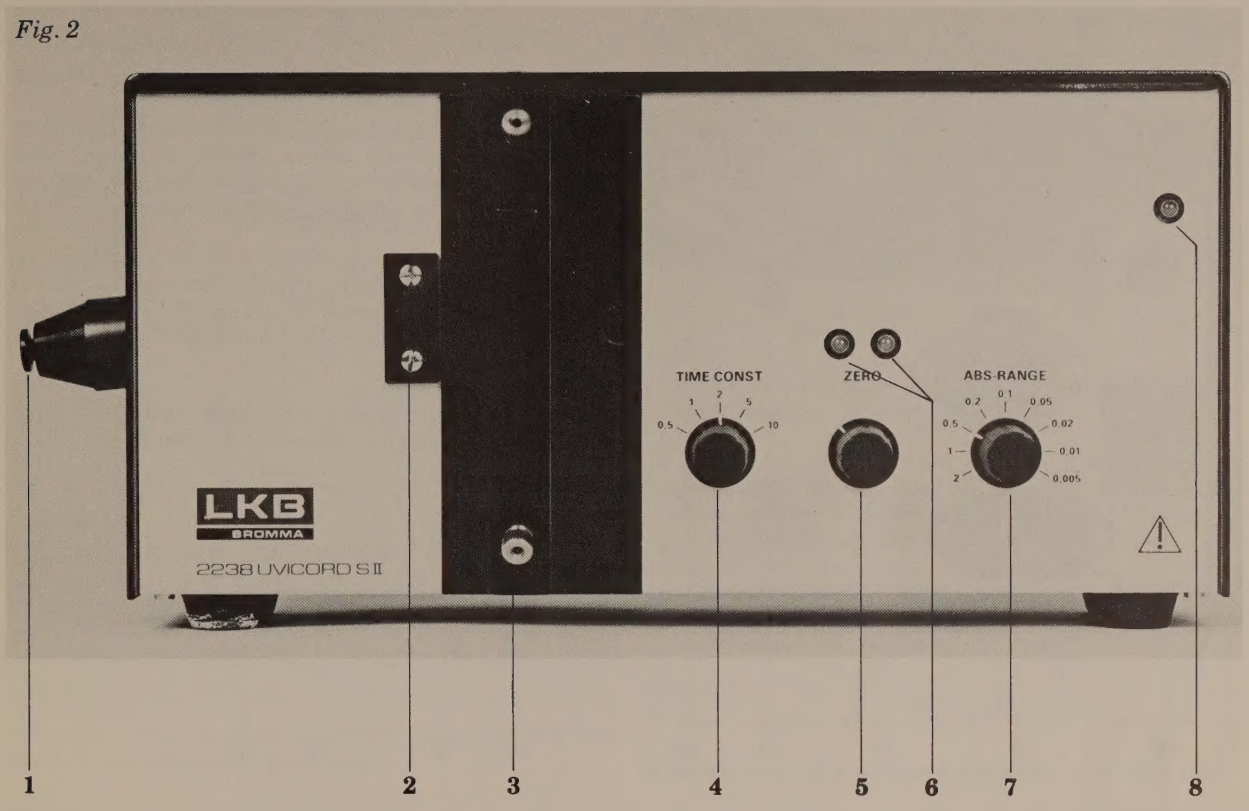


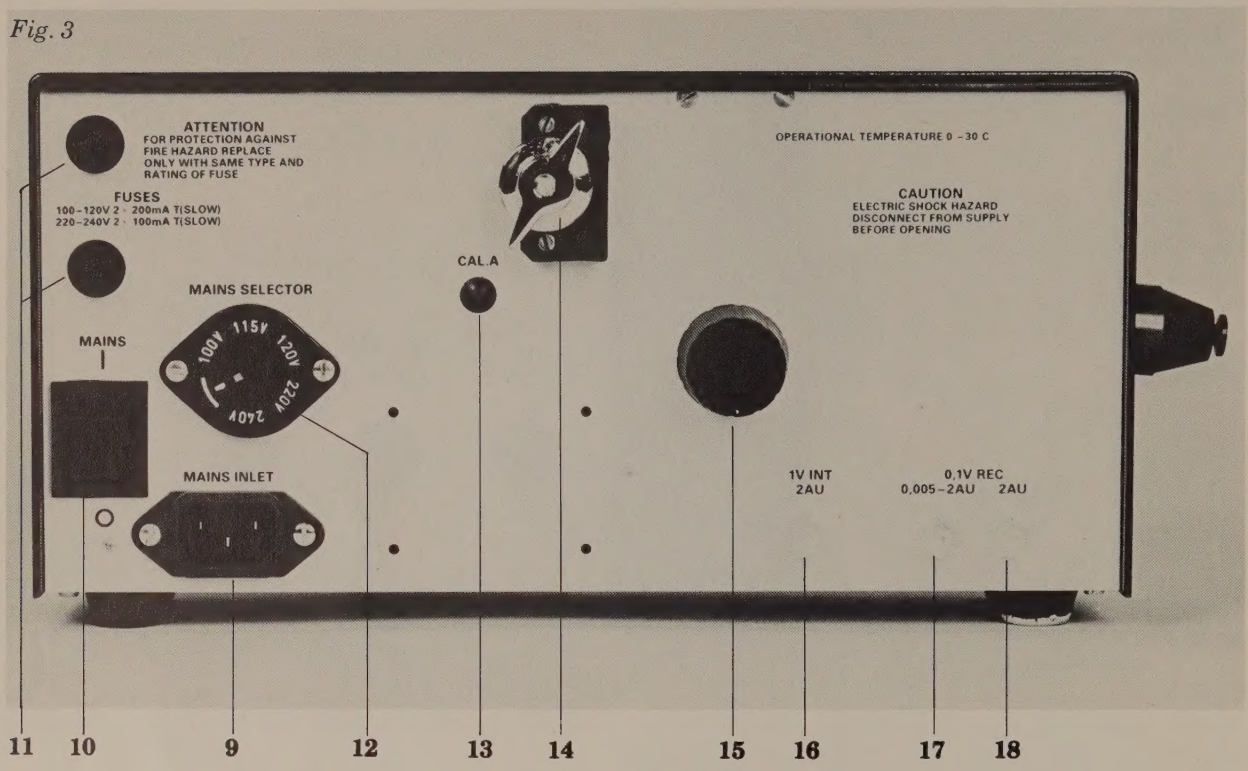
Fig. 2



Front panel

No.	Item	Description
1	**Lamp holder**	The chosen monitoring wavelength determines which lamp shall be used. Each lamp type is identified by a figure (1) or (2) on the end of the lamp. Choice of lamp 1 or 2 is determined by the selected monitoring wavelength.
2	**Filter holder**	The filter holder has a spring-loaded locking mechanism for quick release of the filter. The holder can be inserted into the instrument either way.
3	**Cell holder**	The complete cell holder is quickly removed by unscrewing the knob (15) on the back panel.
4	**Time const. selector**	Selector for the desired time constant (0.5, 1.0, 2.0, 5.0 or 10 s).
5	**Baseline adjust**	Ten-turn potentiometer to adjust the absorbance baseline on the recorder outputs.
6	**Baseline indicators**	Two lamp indicators for remote recorder baseline setting.
7	**Absorbance range selector**	Selector for the desired absorbance range (0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 or 2 AU full scale deflection).
8	**Indicator lamp**	Indicates power is on.

Fig. 3



Rear panel

No.	Item	Description
9	**Mains inlet**	Inlet for the mains cable.
10	**Mains switch**	The Uvicord S II is turned on by pressing the upper half of the rocker switch. The indicator lamp will light to show that mains voltage is on.
11	**Fuse holder**	Main fuses 2 × for 220-240 V, 50 Hz, 100 mA 2 × for 100-120 V, 60 Hz, 200 mA
12	**Mains selector**	Selects mains voltage 110, 115, 120, 220, 240 V.
13	**Calibration potentiometer**	With the calibration potentiometer it is possible to compensate for tolerances in optical pathlength.
14	**Mounting bracket**	For attaching to laboratory rod.
15	**Cell holder release knob**	For quick release and optical alignment of flowcell.
16	**Intergrator output**	Output for integrator 2AUFS =1V.
17	**Absorbance output**	Output for potentiometric recorders (i.e. LKB 2210 and LKB 2065).
18	**Fixed 2 AU output**	2:nd channel output for potentiometric recorder with a dual pen.

III Installation and Maintenance

General precautions

Environmental conditions

The Uvicord S II is designed for use in normal laboratory conditions and should not be installed where the atmosphere is unduly moist, corrosive or contaminated by vapours which may form deposits on components in the optical path.

To minimize drift, the temperature should be kept constant. Thus the Uvicord S II should not be placed in a draught, in direct sunlight or in the vicinity of any apparatus which may cause temperature fluctuations.

NOTE:
The Uvicord II should never be placed near the compressor or in the fan stream of coldboxes or coldrooms

Lamps

Once installed and stabilized the Uvicord S II should not be bumped or tilted. Particular care should be taken to avoid touching the lamp, as this may cause a disturbance in the distribution of lamp material resulting in an undesired shift of light intensity.

WARNING:
Should a lamp accidentally break, remove all Hg and glass to avoid mercury poisoning.

Filters

Special care must be taken when handling interference filters. These filters should not be exposed to temperatures exceeding 60°C, nor should they be allowed to come in contact with any kind of liquid.

Filters should be cleaned with lens cleaning tissue only. Other optical surfaces may be cleaned by wiping the surface with a clean, lint-free cloth moistened with carbon tetrachloride, ethanol, or some other suitable pure solvent.

Before use

Setting up:
When unpacking the instrument please check with the packing list to ensure that all items are included and in good condition.

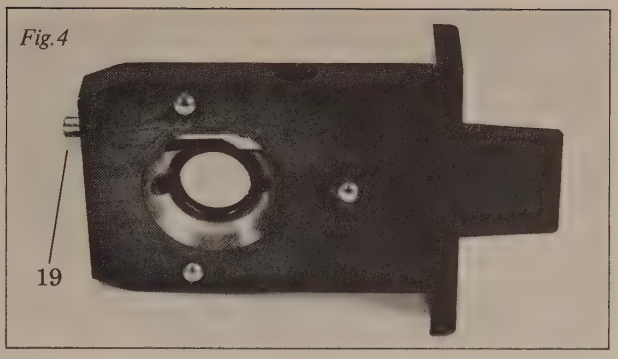
1. Check that the mains selector (Fig. 3:12) is set to the correct mains voltage. To adjust the voltage selector, insert a coin into the slot and turn it until the white mark clicks into place opposite the correct voltage.

2. Check that fuses with correct ratings (as indicated on the rear panel) are inserted in the fuse holders (Fig. 3:11).

3. Check that the mains ON/OFF switch (Fig. 3:10) is in the off position. Connect the mains cable to the mains inlet socket and to the mains supply.

4. Connect the recorder cable to the desired output connectors on the rear.

Fig.4



NOTE:
It is not necessary to use output 17 and 18 (Fig 3) simultaneously.

5. Mount the desired filter into the filter holder (fig. 2:2). Depress the lever (fig. 4:19) and insert the filter gently with the help of forceps. Release the lever (19) to hold the filter firmly in position.

   Always make sure that both sides of the filter are clean (use lens tissue paper to clean) and never touch the surfaces with your fingers.

NOTE:
It doesn't matter which side of the filter faces the lamp.

It doesn't matter which way the filter holder goes in.

6. Insert the appropriate lamp (lamp 1 for 206, 226 or 280 mm, lamp 2 for 254, 313, 365 or 405 mm) into the lampholder (fig. 2:1).

NOTE 1:
If the lamp doesn't ignite, remove and gently rub the glass bulb against a cloth in order to produce static electricity. Re-insert the lamp.

NOTE 2:
Always check that there is a filter in the filter holder, before trying to ignite the lamp.

7. Remove the cell holder (fig. 2:3) by turning the knob (fig. 3:15) on the rear, counter clockwise.

Stabilization time

The lamps normally require a stabilization time of 30 min for work in the absorbance ranges 2A to 0.5A, and 4 hours for work in the ranges 0.2A to 0.005A. It is thus recommended to leave the instrument switched on, unless longer (i.e. weeks) periods of non-use are expected.

IMPORTANT NOTE:
When operating in coldrooms, i.e. at temperatures below 10°C, make sure that the instrument is placed in the coldroom at the desired running temperature 12 hours before a run. This is necessary to lower the temperature of the instrument housing. Once this has been done the stabilization times stated above are valid, i.e. if an instrument is left in a cold room it will have a short stabilization time when it is switched on.

Baseline setting.

1. Adjust the zero position (right hand side of the chart) on the recorder by short-circuiting the input or depressing the zero check knob on the recorder. Select the 100 mV range on the LKB 2210 recorder. The Uvicord S II has a positive output. If using an old LKB 2210 Recorder, Zero position on the recorder is on the left hand side of the chart.

2. Adjust with the zero knob on the recorder.

3. Set the time constant selector at 0.5.

4. Set the absorbance range selector at 2Au.

5. Turn the baseline adjust knob (Fig. 2:5) until both baseline indicator lamps are extinguished. If the left indicator lamp is alight turn the knob to the right and the recorder pen will move to the right. If the right indicator lamp is alight turn the knob to the left.

6. Set the absorbance range selector at 0.5Au, 0.1Au, 0.02Au, 0.005Au and repeat (5) between each absorbance range setting.

7. Set the operating absorbance range.

8. Adjust the Zero point on the recorder to get the baseline (normally 5 to 10 divisions to allow for negative trends).

Selection of time constant.

Set the desired time constant (0.5, 1.0, 2.0, 5 or 10 sec.)

0.5 sec – Fast response for normal conditions.

1.0 or 2 sec – Slow response for supressing short term noise.

5 or 10 sec – Slowest response for maximum suppression of high frequency noise.

NOTE:
If the recorder stands beside the UV-monitor, step 4, 5 and 6 can be omitted, and the desired absorbance range can be set.

IV Operation

The UV-monitor in a chromatography system

A UV-monitor is the simplest and yet the most universal of all chromatography detectors. It can be employed as an analytical instrument with high sensitivity and wide ranging detectability as well as a rather crude monitor for preparative work.

In any chromatography set-up, the UV-monitor must be considered as an integral part, and the chromatographic conditions must be chosen to fit not only the actual separation but also the parameters of the detector. The parameters which must be considered are:

Selection of operating wavelength

When choosing the operating wavelength, one must consider both sample and buffer spectra and concentrations. Generally speaking, operation at short wavelengths increases detectability and sensitivity, i.e. more peaks are seen and smaller amounts are detected.

1. Unknown samples

Unknown samples are best detected at 206 nm, since virtually all biochemical compounds have some UV absorbance at 206 nm and one is therefore assured of not missing any peaks because a compound does not absorb at, for instance, 254 or 280 nm. Furthermore, detection at 206 nm yields much higher sensitivity than 254 or 280 nm since the extinction coefficients for biochemical compounds, at 206 nm, are usually 2 to 200 times greater than those at 254 or 280 nm. This means that trace components which are completely undetected at 254 and 280 can be detected at very small concentrations at 206 nm.

2. Known samples

Known samples are best detected at the wavelength where all the peaks of interest to the chromatographer can be monitored at a satisfactory sample concentration. Whereas any compound is best detected (i.e. lowest concentration necessary) at its extinction maximum, the problem for the chromatographer is to find a wavelength which gives a strong enough extinction by all the compounds which are to be detected. In this respect, 206, 226 nm offers strong advantages over both 254 nm and 280 nm, for particularly protein monitoring as the sample concentrations, when monitored at 206 nm, need only be 1/10th, or less, of the concentrations required to give the same peak height at 254 or 280 nm.

3. Buffer conditions.

In applications where the buffer absorbance at 206 nm is too high the 226 nm wavelength is a perfect alternative.

The light source intensity of Uvicord S II is so high that background absorption due to buffers can be " backed off" up to concentrations corresponding to 0.4 A at 206 and 254 nm with a 2.5 mm cell.

Should the buffer absorption be too strong in a 5 mm cell, a reduction of pathlength down to, for instance, 0,5 mm will give a 10-fold increase in buffer concentration that can be backed off. The amplification obtained by changing from absorbance range 2 A to absorbance range 0.005A would, in this case, still ensure that sensitivity is not lost.

Selection of flow cell

1. Cell volume and influence of volume of connecting tubing.

For both preparative and analytical chromatography it is important to minimize peak broadening due to large mixing volumes in the flow cell and connecting tubing. One should therefore, as a general principle,

choose the shortest possible length of connecting tubing with the smallest possible inner diameter when setting up the detector. Furthermore, the flow cell should have as small a volume as possible; however, bear in mind here the restrictions on cell volume already set by choice of optical pathlength due to buffer background absorption or sensitivity requirements. A variety of flow cells can be obtained (see Appendix C)

2. Optical pathlength.

A range of cells, with optical pathlengths from 0.5 to 5.0 mm, are available (see Appendix B). Selection of appropriate optical pathlength is a means of optimizing sensitivity in accordance with buffer background absorption, sample concentration and the linear dynamic range of the detector.

For applications with a strongly absorbing buffer, one would choose a short pathlength, e.g. 0.5 mm, and compensate for the ensuing sensitivity loss by selecting an absorbance range such as 0.01 or 0.005A.

In cases where the sample concentration can be freely chosen, one should strive to work with as long a pathlength as possible, i.e. 5 mm, in order to minimize noise. Furthermore, one should try to work with an absorbance range which is optimal from a linearity point of view, i.e. 0.005 to 0.5A.

In cases where the sample concentration is low, one should strive for large pathlengths and employ amplified absorbance ranges. For a quantitative discussion or detection limits, see "Selection of Absorbance Range".

In cases where the sample concentrations vary greatly and it is desirable to detect both strongly and weakly absorbing components, one should employ the longest possible pathlength and the highest possible amplification. In combination with this, one should also take advantage of the dual recorder outputs (the 2AU fixed and the selectable absorbance range), so that both large and small peaks can be recorded simultaneously. See "Signal Outputs".

Selection of Absorbance Range

1. Choice of absorbance range

In most cases it is very difficult to determine the best absorbance range a priori. However, knowing an approximate concentration level one can use the Lambert Beer's law:

$A = \varepsilon x l x C$

where ε_1^{λ} = molar extinction coefficient at wavelength λ, measured with a 1 cm optical pathlength (l/mole.cm).

l = optical pathelength in cm.

C = concentrations of sample in moles/liter.

Knowing the approximate concentration level and from a UV-atlas the ε-value, one can determine the approximate absorbance level. One must however, also estimate the dilution factor generated by the chromatography system (usually 5 to 100 times).

An example:

The ε_1^{254}-value for $K_2Cr_2O_7$ taken from a UV-atlas is 4310.

The column dilution factor is estimated to be 10.

The optical pathelength of the flow cell is 5 mm (0.5 cm).

The sample concentration is estimated to be 0.1 mM.

Thus,

$A = 4310 \times 0.5 \times 0.1 \times 10^{-3}$ absorbance units

≈ 0.2155 absorbance units

With a dilution factor of 10 one would then estimate the absorbance to be roughly 0.02A, i.e. the absorbance range 0.05A should be used.

2. Detection limits

Conversely to the above discussion on absorbance ranges, one can estimate what the concentration limit is for a substance, once the following is known:

a) the noise level peak-to-peak with flowing buffer

b) the ε_1^{λ}-value

c) the optical pathlength

d) the dilution factor

The detection limit is defined as the concentration which produces a peak which is distinguishable from baseline noise. The lowest possible detectable signal is normally defined as twice the baseline noise measured peak to peak.

$$\text{Rewriting Lambert Beer's law } C = \frac{A}{l \times \varepsilon_1^{\lambda}}$$

$$\text{Concentration detection limit} = \frac{2 \times \text{noise peak-to peak}}{l \times \varepsilon_1^{\lambda}}$$

Example

With a noise level (with flowing buffer) peak-to-peak of 0.0001A and (ε_1^{254}=4310 ($K_2Cr_2O_7$), dilution factor 10, optical pathlength 0.5 cm, the concentration detection limit

$$= \frac{2 \times 0.0001}{0.5 \times 4310} \times \frac{\text{moles}}{1} \approx 90 \text{ nanomoles/liter}$$

With a dilution factor of 10, ten times as high a concentration is required for sample application i.e. 0.9 u moles/liter

Note that, using 206 nm, where ε_1^{206} for $K_2CR_2O_7$= 10200, would have yielded a concentration detection limit of 0.4 μmoles/liter of $K_2Cr_2O_7$

i.e. at 206 nm the detector is more sensitive for $K_2Cr_2O_7$ than at 254 nm.

3. Calibration

In order to check that the filter has not been degraded (i.e. that its transmission level has not fallen) and to

check that there is no absorbing deposit in the optical path, the instrument can be calibrated against a known standard. Calibration is necessary when comparisons between chromatogram runs are with different pathlengths are used. In this case, one can adjust the calibration to compensate for tolerances in the nominal pathlengths of the flow cell.

The calibration substance recommended is $K_2Cr_2O_7$, dissolved in 0.05N H_2SO_4 which is a spectrum suitable for the three wavelengths: 206, 254 and 280 nm.

$K_2Cr_2O_7$ gives the following ε_1^{λ} -values:
$\varepsilon_1^{206} = 10200$
$\varepsilon_1^{254} = 4310$
$\varepsilon_1^{280} = 3300$

Thus with a 2.5 mm optical pathlength cell, the following concentrations should give a 0.4A deflection:

0.157 mM $K_2Cr_2O_7$ in 0.05N H_2SO_4 at 206 nm
0.371 mM $K_2Cr_2O_7$ in 0.05N H_2SO_4 at 254 nm
0.485 mM $K_2Cr_2O_7$ in 0.05N H_2SO_4 at 278 nm

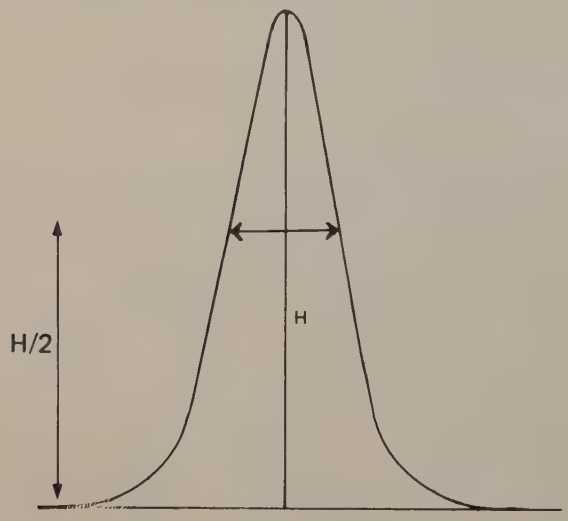
Calibration procedure

Calibrates absorbance output against known standard.

1. Zero the instrument as described in "Baseline setting".
2. Select the 0.5A absorbance range.
3. Fill the flow cell with the appropriate concentration of $K_2Cr_2O_7$, see previous page.
4. Check that the absorbance is 0.4A.
   If not: Adjust the recorder deflection by turning the calibration screw (Fig. 3:13) situated behind the rubber plug) on the rear, until it shows 0.4A.

   If it is impossible to reach an 0.4A level, check that the optical pathway is clear.

   The absorbance scale is now altered to compensate for tolerances in the optical pathlength. This may alter the calibration of % T and absorbance and it is recommended to use any of the alternative outputs for getting high and low sensitivity. See "Signal Outputs".



4. Comparison of chromatography peaks

In order to compare concentrations of chromatographic peaks between runs, the integrated area over the baseline can be used as a measure.

This area measure is best calculated by online computing integrators, (i.e. LKB 2220 Recording Integrator) but can also be calculated manually by multiplying the peak height at H/2 bandwidth of the peak.

Example:
In order to compare concentrations of chromatographic peaks between runs where different optical pathlengths have been employed, one must make sure that calibration has been carried out as indicated in the "Calibration".

5. Factors affecting linearity and quantitative comparisons

The degree of linearity of a UV-monitor is predominantly a function of how monochromatic the light is. For this reason, monitors which employ light sources yielding a line spectrum, such as from a Hg-lamp, will show superior linearity to monitors employing light sources with continuous spectrum, such as deuterium or xenon lamps.

The non-linear behaviour of UV-monitors is caused by stray light, which can be divided into two categories:

a) stray light directly adjacent in wavelength to monitoring wavelength, due to broadband filters or to the bandwidth of grating plus aperture.

b) stray light not adjacent in wavelength to the monitoring wavelength, due to insufficient blocking capacity of filters or grating.

Stray light of type a) influences linearity negatively if the sample spectrum shows a sharp incline or decline over the monitoring wavelength, and non-linearity increases in this case with width of stray light adjacent to the monitoring wavelength.

Note that, if the sample spectrum is flat over the monitoring wavelength, this non-linearity does not appear.

Stray light of type b) influences linearity negatively, particularly if the sample is transparent somewhere in the photo-detector response. The reason for this is that a small portion (i.e. the stray light) of the light giving 100% Transmission (i.e. the I_0 in $A = -\log I/I_0$) will always reach the detector regardless of sample concentration, which evidently means that A cannot reach I_0 and thus that a non-linearity shows at high concentrations.

With regard to the stray light problems, LKB employs both at 206, 254, 313, 365 and 405 nm lamps which give spectral emission lines in combination with filters with excellent blocking capacity.

For quantitative comparisons of known sample peaks, the sample spectrum should be studied to ensure that a non-linear behaviour is not likely to occur.

Fig. 5

Signal outputs

1. 100 mV outputs

There are two 100 mV outputs for use with LKB 2210-recorders, LKB 2065-recorders or similar recorders. One output is for the fixed 2Au (Fig. 3:18) and one for the absorbance range (Fig. 3:17) selected with the range selector (Fig. 2:7).

Fig. (5) shows how to connect either one or both outputs to 2-channel LKB 2210-recorder.

Fig. 6

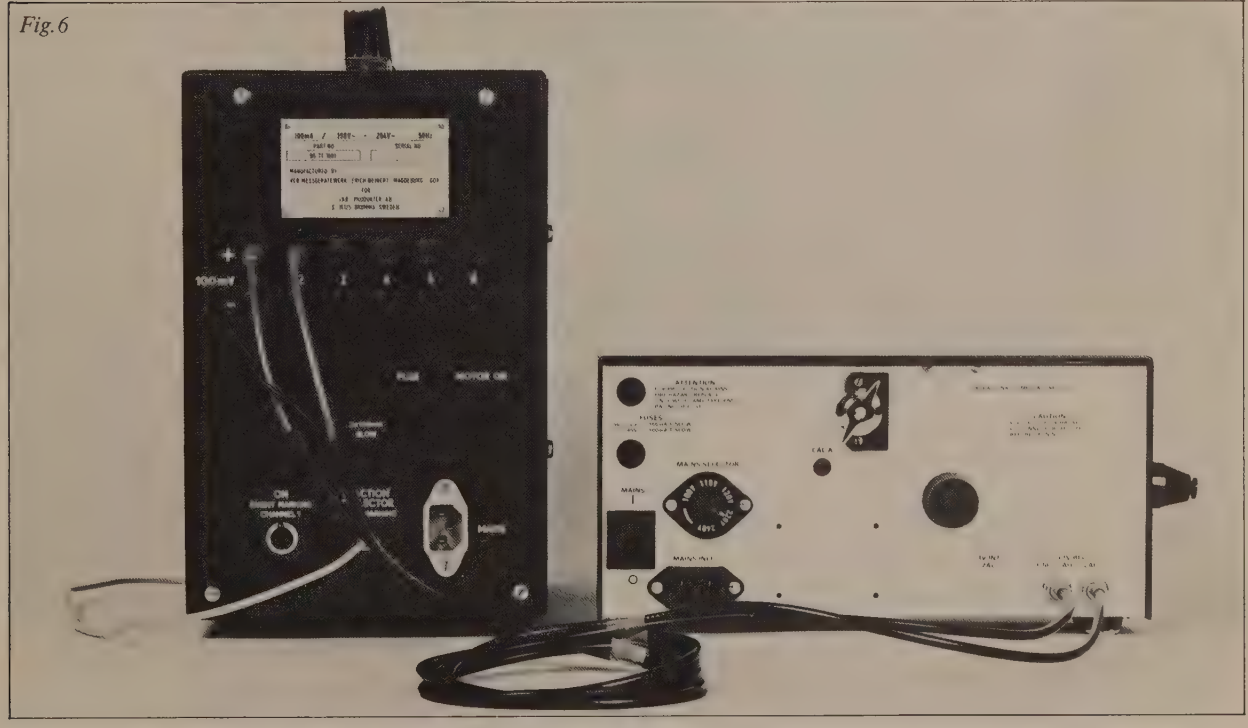
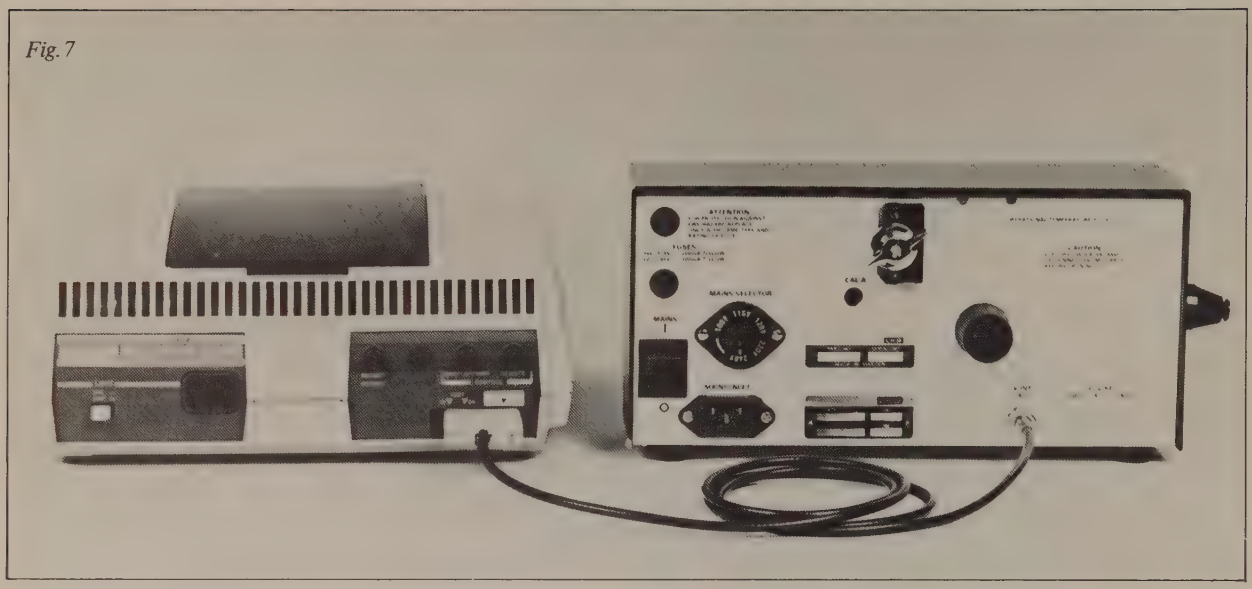


Fig. (6) shows how to connect either one or both outputs to a LKB 2065 6-channel recorder.

Fig. 7



2. Integrator output.

The integrator output yields 1V/2 absorbance units, independent of the selected absorbance range.

Fig. (7) shows how to connect the integrator output to a LKB 2220 integrator. (2220-400 connecting cable)

Appendix A

Technical specification

(A = -log I/I_0 regardless of pathlength)

Operating wavelengths
206, 226, 254, 280, 313, 365 and 405 nm

Absorbance ranges
0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2A

Noise level
<0.0001 Au measured peak-to-peak in a dry cell.

Drift
<0.0005 Au/h at constant temperature.

Time constants
0.5, 1, 2, 5, 10 sec

Flow cells

Standard cell:	Quartz 2.5×5.0 mm rectangular cell (either dimensions can be used as a pathlength) 70 µl cellvolume.
Micro cell:	316 SS (SIS 2343), Teflon® and quarts cell. 2.5 mm pathlength 8 µl cellvolume. Inlet and outlet: 1.59 mm OD and 0.254 mm ID (0.0052″ and 0.010″) 316 SS.
HIFLO cell:	Quartz, 2.5 mm pathlength rectangular cell 4.0 ml cell-volume (includes inlet and outlet volumes) Inlet and outlet 7.0 (±3) mm OD and 4.7 (±2) mm ID.

Recorder outputs
100 mV

Integrator output
1 V/2 Au

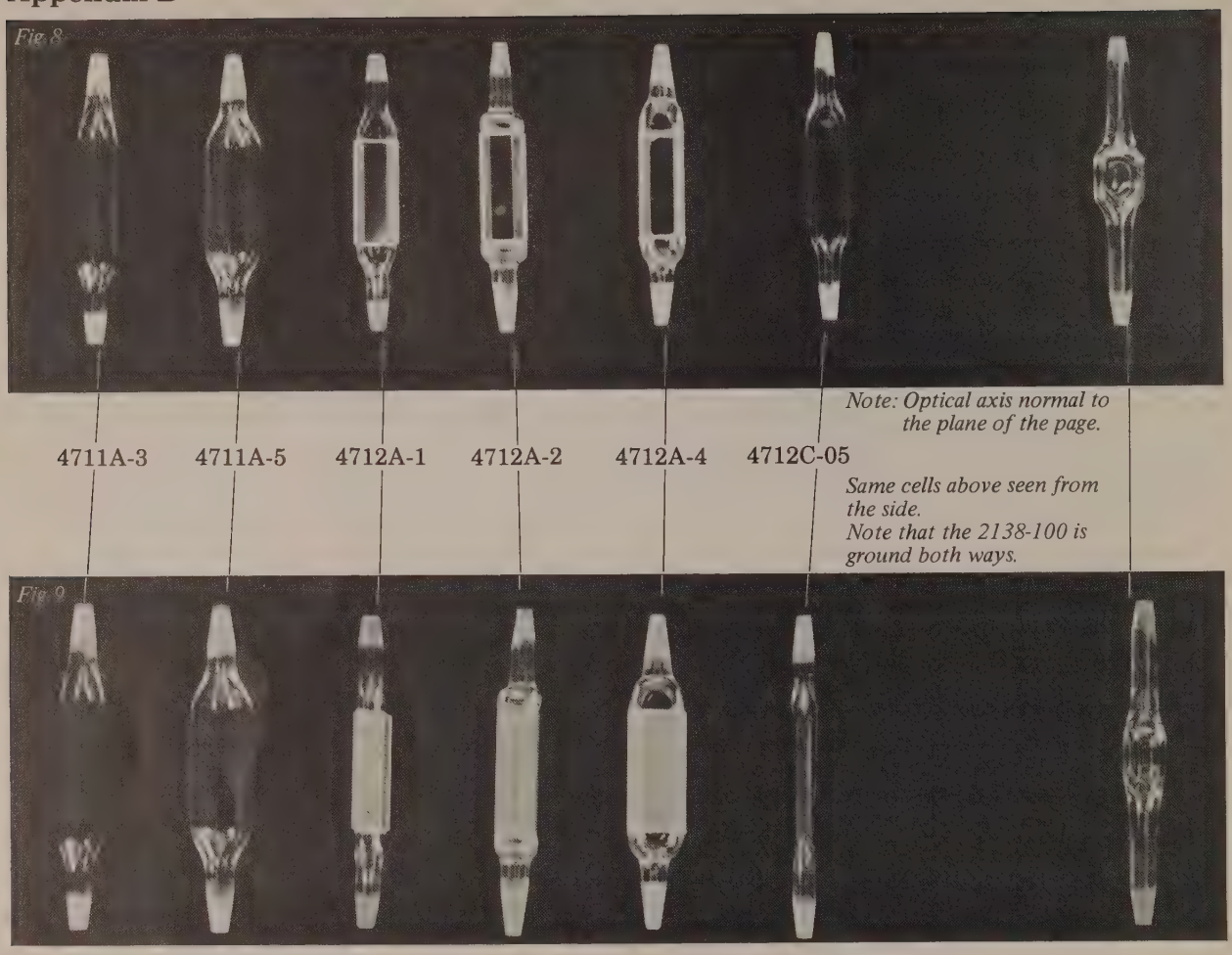
Operating temperatures
0–30°C

Power consumption
20 W

Mains voltage
100–120 V ±10%
220–240 V ±10%
50 or 60 Hz

Dimensions (L×W×H)
313×131×147 mm
Weight 4.3 kg

Appendix B



Optional flow cells

All LKB flow cells are straight flow-through quartz cells, designed to fulfill requirements regarding inertness, ease to change, ease of inspection, independence of flow rate variations and refractive index effects and having a variety of optical pathlength.

Available flow cells are:

Flow cells	Optical pathlength (mm)	Volume (ml)	Cross section	Max. flow rate at 150 cm water head (l/h)
4711A-3	3	0.1	circular	1-2
4711A-5	5	0.3	circular	1-2
4712A-1	1	0.05	rectangular	1-2
4712A-2	2	0.1	rectangular	1-2
4712A-4	4	0.2	rectangular	1-2
4712C-05	0.5	0.03	rectangular	0.2-0.5
2138-100	2.5 or 5.0	0.07	rectangular	1-2

Appendix C

Installation and cleaning of flow cell holder

1. 2138-102 Flow cellholder for LC.

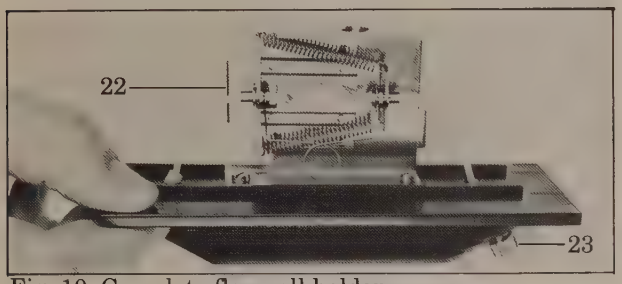


Fig. 10. Complete flow cell holder.

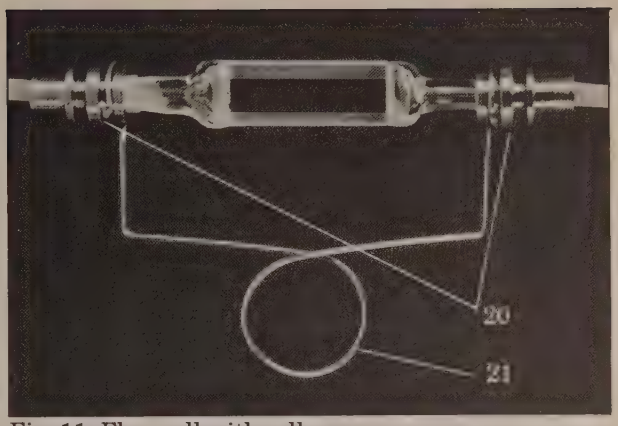
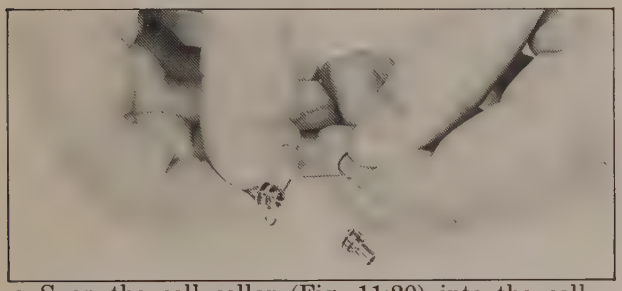
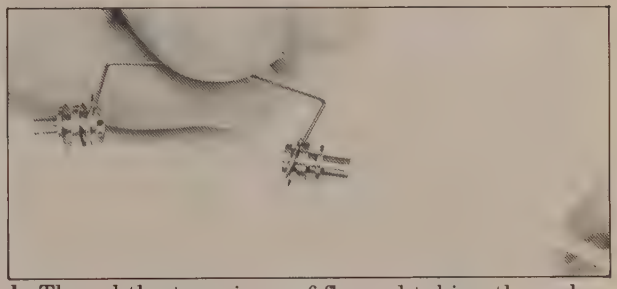


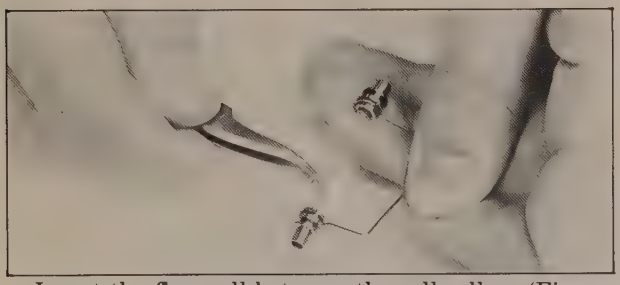
Fig. 11. Flow cell with collar.



a. Snap the cell collar (Fig. 11:20) into the cell holder spring.



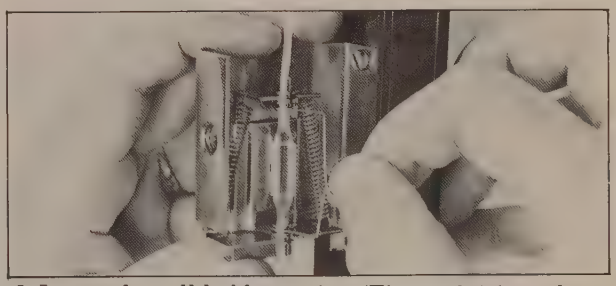
b. Thread the two pieces of flanged tubing through the cell collar (Fig. 11:20).



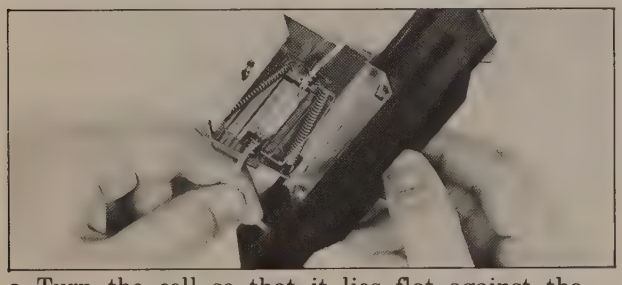
c. Insert the flow cell between the cell collars (Fig. 11:20).

NOTE:
The flow cell should be cleaned before inserting (see cleaning the flow cell).

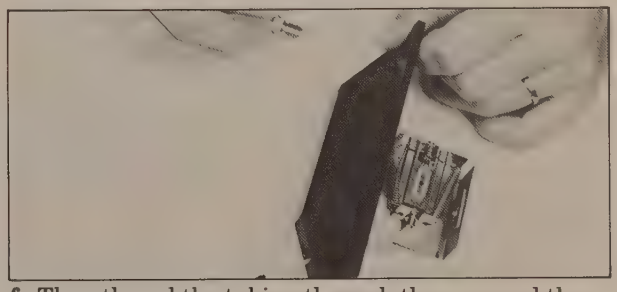
Clean the cell with lens cleaning paper.



d. Insert the cell holder spring (Fig. 11:21) into the spring loaded holder by depressing the lever (Fig. 10:22).



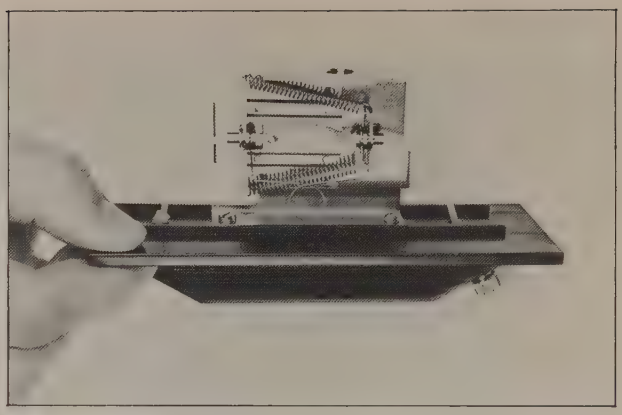
e. Turn the cell so that it lies flat against the aperture hole. Avoid scratching the cell.
Insert the tubing through the inlet and outlet holes of the cell holder (Fig. 11:21).



f. Then thread the tubing through the cone and the fastening screws.

NOTE:
To thread the tubing through the cone and the fastening screw it is usually necessary to unscrew the fastening screws (Fig. 10:23) and remove small slotted plastic cone.

g. Fix the tubing by tightening the fastening screws (Fig. 10:23).



h. Now the flow cell is ready to be inserted in the instrument.
Re-insert the flow cell holder by tightening the knob on the rear panel (Fig. 3:15).

Cleaning of 2138-102 flow cell holder

Clean flow cells are essential to the proper operation of the Uvicord S II. Cells should not be allowed to dry out if they contain liquids with dissolved salts, proteins or other components with low volatility.

If the cell is contaminated proceed as follows:

Remove the cell holder from the Uvicord S II.

Connect a syringe to the outlet tubing and flush the cell with small volumes of 50% (v/v) ethanol in water. Examine the cell from time to time, to check that all the particles have been washed out.

Rinse the cell with about 100 ml of distilled water, and replace it in the Uvicord S II.

Re-connect to the chromatography system.

Most non-particulate contaminants, e.g. denatured proteins, salts, etc. can be removed by flushing the cell with a warm laboratory detergent solution or other appropriate solvent; whichever is used, the cell should then be thoroughly rinsed with distilled water or clean solvent.

Oily deposits, which increase the tendency to trap bubbles, can be removed by rinsing the flow cell first with a non polar solvent (e.g. hexane), then with a polar solvent (e.g. isopropanol), and finally with distilled water. If the flow cell has been used with aqueous solutions, it should be rinsed in the following sequence: warm aqueous laboratory detergent solution, distilled water, polar solvent, non-polar solvent, polar solvent, distilled water.

Persistant contaminants may be removed with cleaning acid (chromic acid), as follows:

Prepare fresh chromic acid by adding 100 ml concentrated sulphuric acid to 3.5 ml of a saturated solution of sodium dichromate.

WARNING:
Chromic acid is extremely corrosive. Dilute spilt acid immediately with a large excess of water.

Remove the cell holder from Uvicord S II.

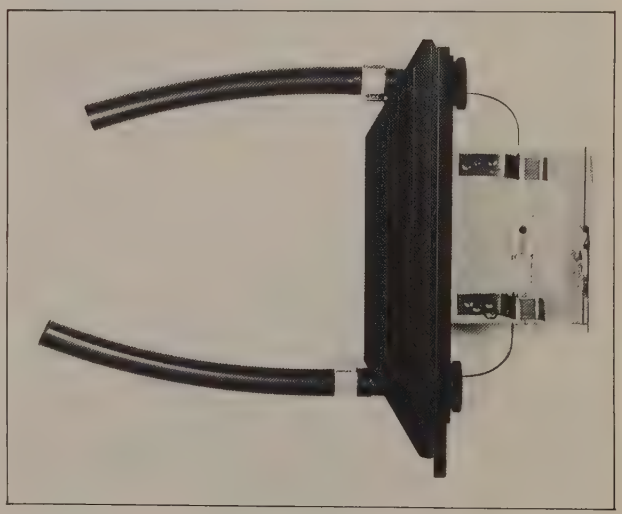
Connect a glass syringe to the outlet side of the cell, and carefully draw chromic acid into the cell. Do not draw acid into the syringe.

Leave the acid in the cell for 10-20 minutes. (Exposure to the acid for several hours, if absolutely necessary, will not harm the cell).

Carefully, remove the cleaning acid, and rinse the cell in the following sequence:
100 ml distilled water, 100 ml 50% (v/v) ethanol in distilled water, 100 ml distilled water.

Replace the cell holder in the Uvicord S II and reconnect to the chromatography system.

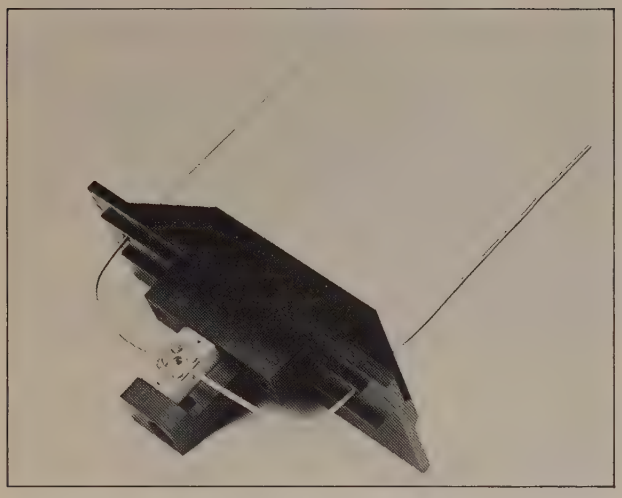
2. 2238-400 Industrial flow cell.



a. The 2238-400 HIFLO-cell is ready to be inserted in the Uvicord S II.

b. Insert the flow cell holder by tightening the knob (Fig. 3:15) on the rear panel.

3. 2138-101 Flow cell holder for HPLC.



a. The 2138-101 Flow cell holder is ready to be inserted in the Uvicord S II.

b. Insert the flow cell holder by tightening the knob (15) on the rear panel.

c. Remove the two rubber stoppers from the tube ends.

d. Flush the cell with at least 50 ml methanol at a rate of 1 ml/min before use.

Detector plumbing

To avoid detrimental effects from dilution, all connections between injector and the cell inlet should be of the zero dead-volume type.

The HPLC cell is not supplied with any connectors because there are several different types used by manufacturers. Follow the instructions supplied with the chosen connector thoroughly and be sure not to overtighten the nuts.

The connecting tubes to the HPLC-cell has an ID of only 0.25 mm (0.01"). The bandbroadening in such small tubing is very little compared with the bandbroadening in the HPLC column itself. Try to connect the column outlet directly to the HPLC cell inlet. The pressure from the column outlet is normally close to the environments, thus it is often appropriate to use Teflon ferrules when connecting the column outlet to the cell.

If for any reason it is necessary to keep a ceratin pressure in the HPLC cell e.g. to avoid bubble formation in the cell, the best way is to keep the outlet several meters above the cell. Connect a wide teflon tubing to the outlet, (upper tube on the cell) and arrange the waste vessel high. This arrangement will also maintain the pressure in the cell even if the flow is stopped and will also be insensitive to flow fluctuations caused by the HPLC pump.

During installation of Uvicord S II or later it may be necessary to cut the stainless steel tubing. This is best done in the same way as one cuts a glass tube. Make a score with a file and bend the tube at the scored point several times in opposite directions until the tube breaks. It is also advisable to take away the burrs around the break with a file but be careful not to file across the opening to prevent filings from falling into the tube.

Lack of flow through the detector

If lack of flow from the detector outlet is noted, first confirm that flow is coming out from the column.

If flow is coming out from the column the blockage is in the cell or connecting tubes.

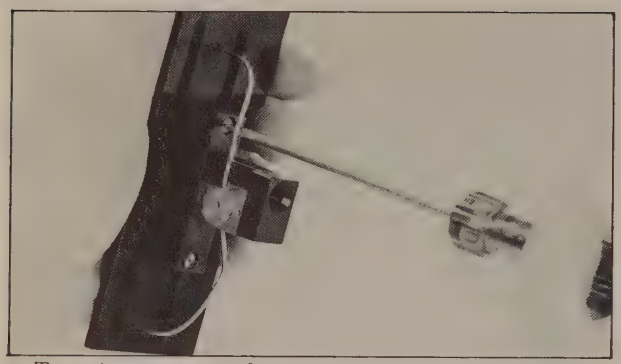
Begin by attaching the pump to the cell outlet and try to remove the obstruction by using reverse flow through the cell at low pressure. A back pressure of not more than 50 bar (725 psi) should be put on the cell. If back flushing is not succesful remove the quartz window on one side of the cell (see "Cleaning of flow cell") and try again.

If the solvent now flows out through the cell the obstruction must be located in the inlet to the cell. If the blockage remains it will be located in the outlet tube.

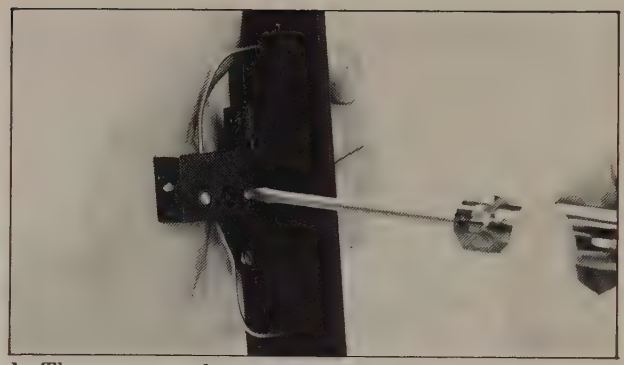
Cut either the inlet or outlet tubing (whichever contains the blockage) close to the flow cell allowing sufficient tubing to remain for reconnection. Assemble the cell and verify that the obstruction is now removed by feeding solvent from the pump through the cell.

If the blockage still remains the cell assembly with connecting tubes has to be changed. Contact your LKB service representative.

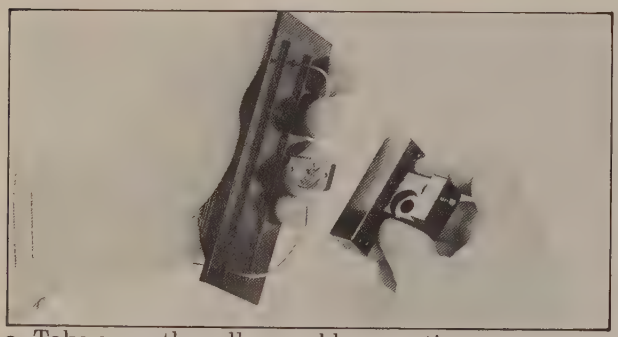
Cleaning of 2138-101 flow cell



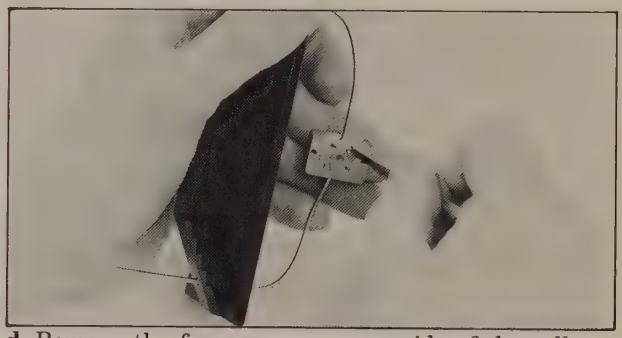
a. To gain access to the quartz windows first remove the two screws holding the cell assembly in the cell holder.



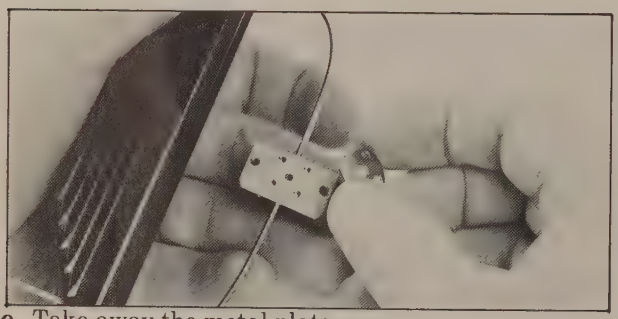
b. Then remove the two screws holding the cell assembly mounting from the handle.



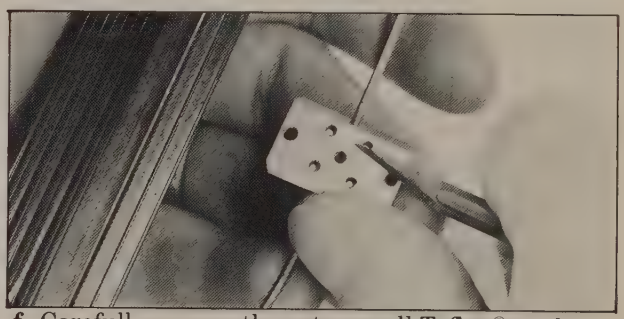
c. Take away the cell assembly mounting.



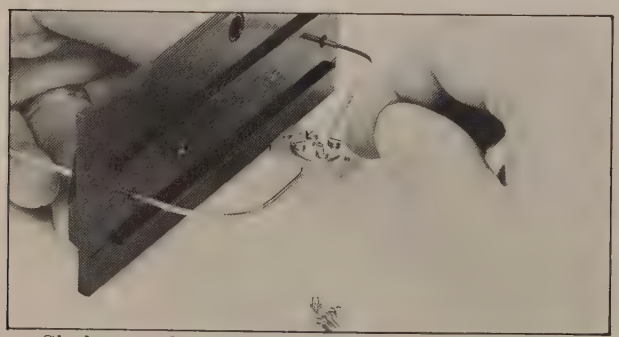
d. Remove the four screws on one side of the cell assembly.



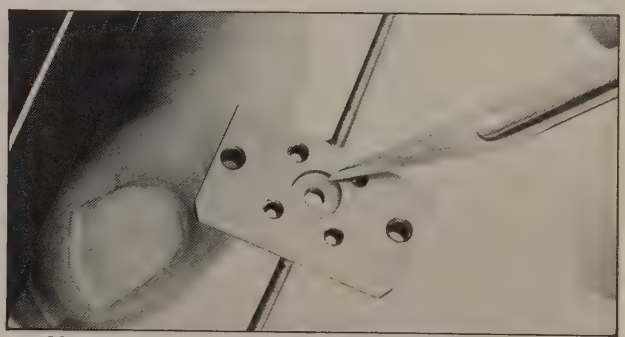
e. Take away the metal plate.



f. Carefully remove the outer small Teflon® washer.



g. Shake out the quartz window.



h. Check the inner teflon washer for damage. If damaged, remove with a knife and replace. Continue in the same way with the opposite window.

i. If the bore of the assembly contains any visible particles it can be cleaned in an ultrasonic bath with alcohol or simply by flushing with a syringe.

j. Wiping with tissue or swabbing with alcohol is usually sufficient to clean the windows. Replace the cleaned window in its slot on top of the bottom washer and place the top washer as before.

k. Replace the metal plate and lightly tighten the plate on the cell. Quartz is brittle and care should be taken in handling it and in securing the windows. Lightly tighten the screws that are diagonally opposite.

l. Repeat for the other two screws and so on until the four screws are firmly fastened. Pass solvent through the flow cell with a syringe and check for leaks. Mount the cell assembly in the cell holder and secure with the two larger screws.

4. Prevention of microbial growth in the chromatography system

The risk of microbial growth is slight when the system is in use, but high when the system is standing idle. Antimicrobial agents may be used as a pre-run rinse. The agent chosen should not interact either with the sample or with the gel. Whichever antimicrobial is used, at least one column volume of buffer should pass through the monitor flow cell before the baseline is adjusted.

Sodium azide (NaN_3), 0.02%, does not interact noticeably with proteins or carbohydrates, and does not change their chromatographic behaviour. It interferes with fluorescent marking of proteins and the anthrone reaction, and inhibits certain enzymes.

Chloretone (trichlorobutanol) 0.05%, is only effective in weakly acidic solutions.

Merthiolate (Thimerosal, ethylmercuric thiosalicylate), 0.005%, is moste effective in weakly acidic solutions. It binds to, and can inactivate, compounds containing thiol groups.

Hibitane (Chlorhexidine), 0.002%, is very effective and is incompatible with only a few substances.

Phenylmercuric salts (acetate, borate, nitrate) 0.001 to 0.01%, are effective only in weakly alkaline solutions.

Cloroform, butanol and toluene are not recommended if the chromatography system contains plastic materials, or when using certain dextrain gels. They are effective only in very concentrated solutions.

5. Prevention of bubble formation

The design of the LKB flow cells eliminates the problem of bubbles being trapped in the optical path.

In order to avoid bubble formation totally, one should:

use only properly degassed buffers.

NOTE:
Always let the direction of flow through the flow cell run from the bottom upwards (with the exception of density gradient runs).

Let the buffer temperature be slightly higher (3-5°C) than the column temperature, which means that the buffer will be cooled in the column, thus increasing its capacity to absorb gases.

If the bubble, in spite of these measures, should form, it is normally flushed out immediately and will only cause a spike on the recorder chart.

If the above measures are not taken and a bubble forms and stays in the flow cell, it is easily removed by applying pressure to the outlet tubing.

Appendix D

Basic principles in absorption spectrophotometry

Light

Light is an electromagnetic wave motion, the physical, chemical and physiological effects of which depend on the wavelength.

The wavelength is measured in units called Ångströms, named after a Swedish physicist, and abbreviated Å. 1 Å is defined as 1/10.000.000 of 1 mm or 10^{-10}m.

Other frequently used units are millimicron and nanometer, abbreviated mu and nm. Both these units have exactly the same value. They are 10 times as large as the Ångström unit, i.e. 1 mμ=1 nm = 10 Å or 10^{-9}m.

The human eye is sensitive to light with wavelengths between approximately 400 and 750 nm. The lower limit corresponds to violet, the upper limit to red light, and in between are distributed the other colours of the spectrum. If all these wavelengths occur together in the relative abundance given by daylight, one speaks of white light. Outside this range of visible light, there are the ultraviolet (lower than 400 nm) and infrared (higher than 750 nm).

Spectrum

A spectrum is a picture or curve which shows how the intensity of a light source varies with the wave lengths. A spectrum is said to be continuous if all wavelengths within a certain range exist (sunlight, light from incandescent lamps). A spectrum is said to be discontinuous (to be a line spectrum) if only discrete wavelengths exist, with dark fields in between (light from discharge lamps). There are also lamps giving superimposed continuous and discontinuous spectra.

Light filters

Filters are made of specially prepared glass (foil contained between glass plates) or cuvettes holding special solutions or gases, which have the ability to absorb (block) some wavelength regions and transmit others. Thus, filters provide a very simple way of isolating a certain colour.

Filtered light from a lamp with a continuous spectrum is a mixture of many wavelengths (polychromatic). On the other hand, a mercury lamp (having a line spectrum) in conjunction with suitable filters can give light of one single wavelength, or monochromatic light. This wavelength, however, cannot be freely chosen.

Light absorption

When light passes through a medium, a loss of intensity usually occurs. This is called light absorption. The light absorption for a media varies considerably with the wavelength. A picture or a curve showing how the light absorption of a substance varies with the wavelength is called its absorption spectrum. Examples of such curves are shown in fig. a. where the left-hand curve refers to formyl-pteroyl-glutamic acid, the middle one to coproporphyrin, and the right hand one to carotene.

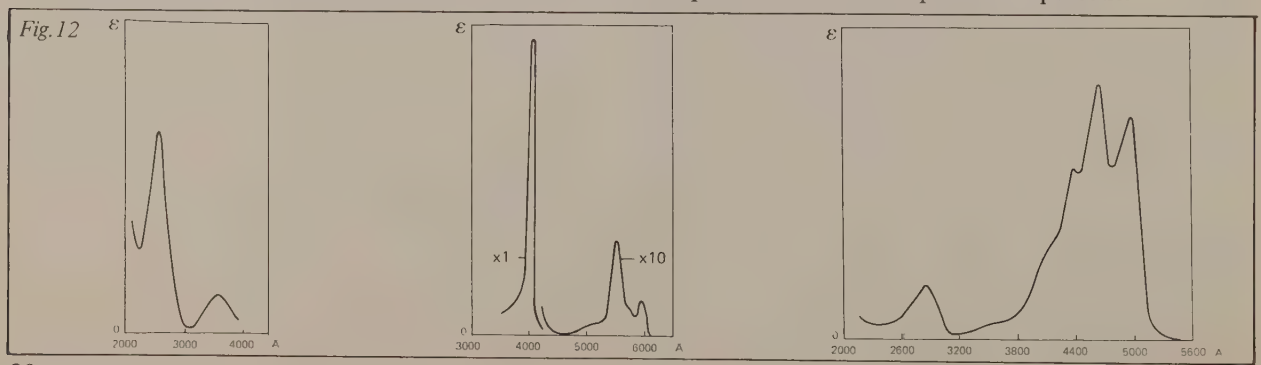
Measurement of light absorption at different wavelengths is called absorption spectrophotometry. As can be seen in fig. 12, absorption spectra are characteristic of the compounds, and recording of such spectra is thus an important aid in identifying chemical compounds.

On the other hand, by measuring the absorption at one single wavelength, one can determine the concentration of a substance provided that no other substances in the sample absorb light at this particular wavelength. It is then better to choose a wavelength which corresponds to a maximum (peak) in the absorption spectrum, partly because there is a maximum in sensitivity at such a point, partly because the absorption is almost independent of the wavelength in a small region at the top of the peak. As a general rule, one can say that the light used for measurement should have a bandwidth appreciably smaller than the width of the absorption band. Thus, the narrower the absorption band, the smaller the required bandwidth, fig. 13. Of course it is possible to perform measurements on the slope of an absorption-peak, but a very small bandwidth is then required.

Definitions in absorption measurements

In absorption measurements it is essential to be familiar with the concepts in current use, which are: The transmission (transmittance), T, of a sample at a certain wavelength which is defined as that fraction of the incident intensity which is transmitted by the sample. It can also be expressed in percent.

Fig. 12



The *cell thickness,* d, refers to the internal measurement of the cell (the sample thickness) and in thus the distance which the light has to travel in the absorbing medium, it should be given in cm.

The *extinction (absorbance, optical density),* E, is defined as the Briggsian logarithm of the ratio I_o/I between the incident and transmitted light intensities.The extinction is proportional to the concentration of the absorbing substance and to the cell thickness. Thus, at a constant cross-sectional area for the light beam, the extinction is a measure of the mass of absorbing material in the path of the light flux.

The *molar extinction coefficient (molar absorptivity),* ε, is defined as the extinction that would be obtained if the concentration were 1 mole per liter and the cell thickness 1 cm.

The *specific extinction absorptivity),* k, is defined as the extinction that would be obtained if the concentration is other than 1 mole per liter and the cell thickness 1 cm. The value depends on the concentration unit chosen. Specifically, if the concentration is measured in grams per 100 ml, the specific extinction is often written, not quite logically*), $\frac{E^{1\%}}{1\ cm}$.

Specific extinctions are used instead of molar extinctions in cases where the molecular weight is unknown or known with an accuracy lower than the one with which the extinction can be measured.

The Lambert-Beer's law

The mathematical relationship between the quantities defined above is expressed by Lambert-Beer's law:

$$I = I_o - 10^{-kcd} = I_o \times 10^{-E}$$

or in other form:

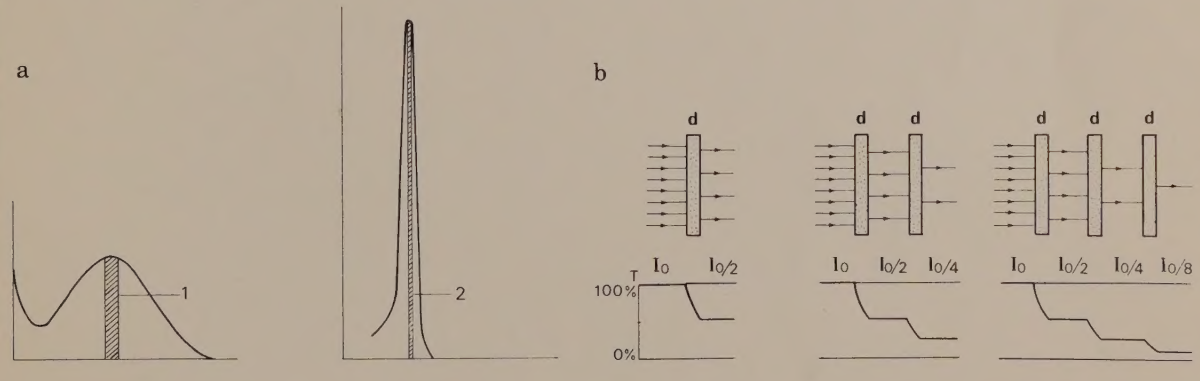
$$E = kcd = \log I_o/I$$

When c is measured in moles per liter, k becomes the molar extinction, E.

The implications of Lambert-Beer's law are as follows. The relative attenuation which affects the light passing through a medium is independent of the incident light intensity. Consequently, one and the same sample absorbs appreciably more light from a strong source than from a weak one. The phenomenon is illustrated in Fig. 13b, where three identical absorbing plates have been placed in series. The first plate absorbs 50% of the incident light I_o, the second plate absorbs 50% of what has passed the second plate, $I_o/4$. Consequently, the extinction varies linearly with the amount of material in the path of the light, while the transmission does not.

*) A concentration of 1% strictly means that the solution contains 1 gram of solute for 99 grams of solvent. However, most authors giving concentrations in per cent in connection with extinction measurements probably have in mind the unit 1 gram per 100 ml of solution. Thus it seems to be agreed upon within wide circles that the unit "mg %" actually means 1 mg per 100 ml. Lambert-Beer's law has absolute validity only for concentration units based on volume.

Fig. 13



LKB

THE INCENTIVE GROUP

LKB-Produkter AB, Box 305, S-161 26 Bromma, Sweden

Stockholm Antwerp Athens (Middle East) Bangkok Copenhagen London Lucerne Madras Moscow Munich Paris Rome Turku Vienna Washington Zoetermeer

Representatives in 60 countries